Oxidative stress in pericardial fluid and plasma and its association with ventricular function

Jose Luis Vukasovic[a,*], Francisco Moraga[b], Guillermo Díaz-Araya[b], Eduardo Turner[c], Mario Chiong[b], Polentzi Uriarte[c], Fernando Florenzano[a], Sergio Lavandero[b,*]

[a] *Department of Cardiovascular Diseases, Faculty of Medicine, University of Chile. Av. Salvador 364 Santiago, Chile*
[b] *Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Olivos 1007, Santiago 6640750 Chile*
[c] *National Thorax Institute, José Miguel Infante 717, Santiago, Chile*

Abstract

There are no studies evaluating oxidative stress markers both in pericardial fluid and plasma and whether they correlate with cardiac function indexes. The purpose of the study was to investigate whether oxidative stress markers in pericardial fluid and plasma are associated with left ventricular function. *Methods and results:* Twenty-eight consecutive patients (class I or II NYHA) scheduled for myocardial revascularization, valve replacement, valve repair or closure of atrial septal defect. Plasma and pericardial fluid were collected and malondialdehyde, catalase, superoxide dismutase and glutathione peroxidase were determined. Left ventricular ejection fraction, left ventricular end diastolic diameter and left ventricular end systolic diameter were determined as echocardiographic indexes of ventricular function.

We found that oxidative stress determined by a simple malondialdehyde (MDA) assay, correlated in plasma and pericardial fluid, and this parameter was associated with left ventricular end systolic diameter. *Conclusion:* Plasma and pericardial fluid malondialdehyde levels can be used as an early marker of ventricular dysfunction.

Keywords: Pericardial fluid; Oxidative stress; Malondialdehyde; Antioxidant enzymes; Ventricular function; Heart failure

1. Introduction

Evidences from different sources suggest that an excessive increase in reactive oxygen species contributes to the pathogenesis of a variety of cardiovascular diseases [1,2]. Under normal conditions, there is a fine balance between reactive oxygen species and a variety of antioxidant defense systems. Any alteration in this balance in favor of reactive oxygen species causes an increase in oxidative stress and initiate cellular changes that may lead to heart failure [3]. Lipid peroxidation generates several unsaturated aldehydes, such as malondialdehyde and 4-OH-nonenal, which may interact with and modify the function of other molecules that are of biological importance. Although heart failure is a state of generalized oxidative stress, the resultant spectrum of saturated and unsaturated aldehydes has not been systematically characterized in this condition in both plasma and pericardial fluid. However, it cannot be ruled out that malondialdehyde is only a marker for increased myocardial damage in these patients.

Oxidative stress has traditionally been evaluated by measuring plasma levels of malondialdehyde and the assessment of antioxidant enzymes such as catalase, superoxide dismutase and glutathione peroxidase [3]. However, no definite information is available concerning the actual relationship of oxidative stress assessed by these methods in plasma with what is actually taking place at the myocardial level. Pericardial fluid is not merely an ultrafiltrate of plasma, but also a transudate from the cardiac interstitium, reflecting the composition of cardiac interstitium and the production and release of macromolecules in normal and diseased myocardium [4]. We determined pericardial fluid malondialdehyde levels as well as their correlations with plasma malondialdehyde levels

* Corresponding authors. Jose Luis Vukasovic is to be contacted at Department of Cardiovascular Diseases, Faculty of Medicine, University of Chile. Av. Salvador 364 Santiago, Chile. Fax: +56-2-269-6263. Sergio Lavandero, Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Olivos 1007, Santiago 6640750 Chile. Fax: +56-2-737-8920.

E-mail addresses: vukasovic@terra.cl (J.L. Vukasovic), slavander@uchile.cl (S. Lavandero).

and different parameters of ventricular function in an unselected group of patients undergoing open heart surgery. In addition, circulating catalase, superoxide dismutase and glutathione peroxidase activities were also evaluated.

2. Methods

2.1. Patients

We included 28 consecutive patients scheduled for myocardial revascularization, valve replacement, valve repair or closure of atrial septal defect. All patients signed an informed consent approved by our Institutional Review Board and Ethics Committee. Patients with acute coronary syndromes, previous myocardial infarction or previous cardiac surgery were excluded. No patient was taking allopurinol or receiving antioxidant vitamins for cardiac protection.

The clinical characteristics of patients are described in Table 1.

2.2. Blood and pericardial fluid samples

The blood samples were obtained by venipuncture at the time of opening the pericardial cavity but before the initiation of cardiopulmonary bypass, while pericardium fluid samples were obtained after opening the pericardial cavity and before administering heparin.

Blood was centrifuged and plasma was stored at −20 °C. The erythrocytes were washed three times with saline solution, homogenized, centrifuged in the same manner. Cell lysates were prepared by adding 0.1 ml cell suspension to 0.4 ml distilled water and stored at −20 °C. Pericardial fluids were centrifuged and stored at −20 °C until analysis.

2.3. Malondialdehyde measurement

Lipid peroxide formation (*malondialdehyde* assay) was determined by examining the content of thiobarbituric acid reactive substances [5]. Commercially available malondialdehyde was used as standard.

Table 1
Clinical characteristics of patients ($n=28$) of cardiothoracic surgery

Age (years)	52 ± 17
Men (%)	50
Etiology (*n*)	
Ischemic	8
Mitral regurgitation	13
Aortic insufficiency	3
Aortic stenosis	1
Atrial septal defects	3
Echocardiographic indexes	
Left ventricular ejection fraction (%)	61 ± 10
Left ventricular end diastolic diameter (mm)	57 ± 11
Left ventricular end systolic diameter (mm)	38 ± 10
Functional capacity according to NYHA (*n*)	
I	9
II	13
III	6

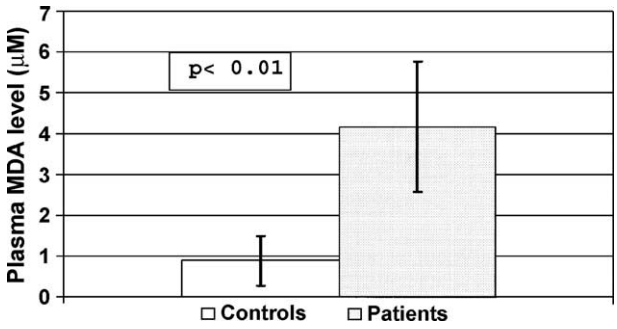

Fig. 1. Differences in plasma malondialdehyde (MDA) levels between patients with dilated left ventricle and controls.

2.4. Antioxidant enzyme activity determination

Superoxide dismutase activity was assayed as described by Misra and Fridovich [6] and expressed as unit (U) per mg hemoglobin. Catalase activity was determined as described by Beers and Sizer [7] and its activity was expressed as mmol of H_2O_2 per min (U) per g hemoglobin. Glutathione peroxidase activity was determined according to Paglia and Valentine [8] and expressed as nmol of NADPH oxidized per min (U) per g of hemoglobin.

2.5. Left ventricular function evaluation

Left ventricular ejection fraction, *left ventricular* end diastolic diameter and *left ventricular* end systolic diameter were determined as echocardiographic indexes of ventricular function. Echocardiographic evaluation was performed before surgery, within 7 days.

Oxidative stress parameters were compared with a control group of 15 healthy, age- and gender-matched volunteers. They were all asymptomatic, with a normal medical history and normal physical examination. Control subjects were excluded if they had any known coronary risk factor, if they were taking any medication, vitamin supplements, antioxidants, or if they drank alcohol on a regular basis.

2.6. Statistical analysis

Results are presented as mean ± standard deviation. Student's *t*-test assuming equal variances was used to assess differences in continuous variables for unpaired data (patients ands controls).

Linear regression analysis was performed to assess the relationship between echocardiographic indexes and values of malondialdehyde, catalase, superoxide dismutase and glutathione peroxidase in plasma and values of pericardial malondialdehyde.

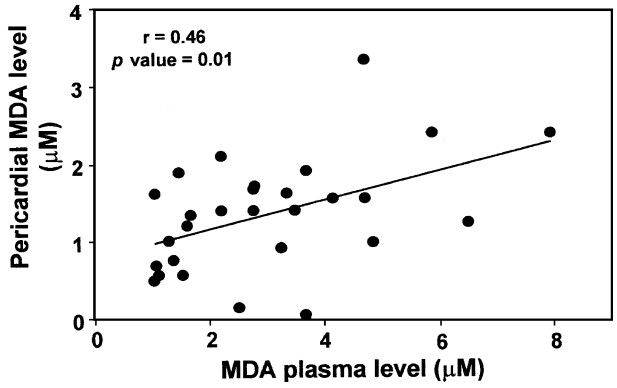

Fig. 2. Correlation between pericardial fluid and plasma malondialdehyde (MDA) levels.

The association of left ventricular systolic diameter and etiology, evolution time, functional capacity and malondialdehyde in plasma and pericardium were evaluated by using a univariate analysis. Afterwards, the covariables of left ventricular systolic diameter were studied using a multiple linear regression analysis by a stepwise forward-conditional procedure. The left ventricular systolic diameter was the dependant variable and age, evolution time, etiology, functional capacity and malondialdehyde in plasma and pericardium were the independent variables.

Statistical analyses were performed using SPSS software version 10.0 (SPSS, Chicago, IL).

A p value <0.05 was considered significant.

3. Results

Patients with increase left ventricular systolic diameter had a significant elevation in MDA plasma levels compared with controls (4.2 ± 2.1 vs. 0.88 ± 0.2 μM value <0.01; Fig. 1).

The univariate analysis showed a significant association of left ventricular systolic diameter with functional capacity (NYHA) and malondialdehyde in plasma and pericardium

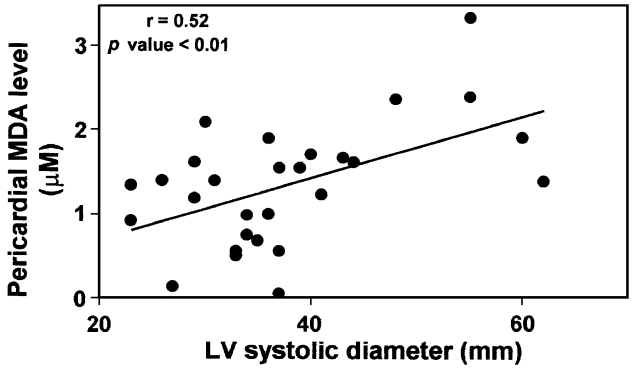

Fig. 3. Correlation between pericardial fluid malondialdehyde (MDA) levels and left ventricular (LV) systolic diameter.

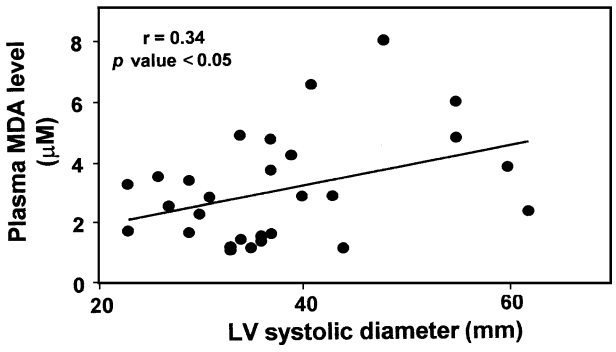

Fig. 4. Correlation between plasma malondialdehyde (MDA) levels and left ventricular (LV) systolic diameter.

($p<0.05$). On multilinear regression analysis only functional capacity and malondialdehyde levels remained as independent co-variables associated with left ventricular systolic diameter ($p<0.05$).

A linear correlation was found between plasma and pericardial fluid of malondialdehyde concentrations ($r=0.46$, p value <0.01; Fig. 2), although the malondialdehyde levels in the plasma were significantly higher than in the pericardial fluid (2.89 ± 1.50 vs. 1.38 ± 0.60 μM, $p<0.01$).

The correlation of left ventricular systolic diameter and malondialdehyde in pericardial fluid was $r=0.52$ (p value <0.01; Fig 3).

In addition, a trend was observed between left ventricular systolic diameters and plasma malondialdehyde ($r=0.34$, p value <0.05; Fig. 4).

Also a significant correlation was found between left ventricular diastolic diameter and malondialdehyde in pericardial fluid $r=0.47$ (p value <0.01).

For antioxidant enzymes (catalase, superoxide dismutase and glutathione peroxidase), no associations were found between circulating levels and echocardiographic indexes (left ventricular diastolic and left ventricular systolic diameters).

4. Discussion

There is progressive evidence supporting the view that levels of different substances in the pericardial fluid correlate well with their level in the cardiac interstitium [9]. On this basis, we postulated that malondialdehyde pericardium concentrations might be an indirect index of oxidative stress at the myocardial level. This hypothesis was supported by the results of our data, indicating a significant correlation between ventricular function markers such as: left ventricular diastolic and systolic dimensions and malondialdehyde concentrations measured in the pericardial fluid.

Two lines of explanations could be postulated for the relationship found between left ventricular function and oxidative stress in the pericardial fluid. One is that over-

stretching of the myocardium leads to enhanced generation of reactive oxygen species, with a consequent induction of myocardial remodeling; this process has been related with an apoptosis mechanism [10]. Further evidence that reactive oxygen species are involved in pathological myocardial remodeling comes from studies in which strategies to reduce reactive oxygen species, including the use of antioxidant vitamins, have exerted beneficial effects in animal models of pressure overload and myocardial infarction [11,12].

An alternative explanation could be that the higher level of oxidative stress observed in patients with dilated ventricles is a reflection only of a severe myocardial damage, hypothesis that cannot be completely excluded with the present date.

In addition we found that patients with dilated left ventricles had a significant increase in plasma malondialdehyde (MDA) levels compared with controls. This finding further supports the idea that patients with dilated ventricles have a higher level of oxidative stress, issue that has been previously reported [13]. We did not observe changes in the activities of the antioxidant enzymatic system conformed by catalase, superoxide dismutase and glutathione peroxidase. The absence of response probably is related to the relatively well-preserved ventricular function found in the majority of our patients. In the literature, modifications on antioxidant enzymes have been reported only in patients with moderate or severe cardiac failure [13] or in heart transplanted subjects [14].

In relation with previous studies of reactive oxygen species in the pericardium, Mallat et al. [15] studied other marker of oxidative stress, the 8-*iso*-Prostaglandin F_2, and also reported a linear correlation between oxidative stress in the pericardium fluid and the ventricular systolic and diastolic dimensions. 8-*iso*-Prostaglandin F_2, was used to evaluate oxidative stress because the authors considered that measurement of malondialdehyde by the thiobarbituric acid-reacting substances assay, suffers from lack of specificity and therefore it is an inaccurate indicator of oxidant stress in vivo. This observation is not supported by our study, since we corroborated their results in pericardium fluid and also noted a significant correlation between plasma and pericardium fluid levels with clear trend towards a relationship between ventricular dimensions and malondialdehyde in the plasma.

Our results suggest that plasma malondialdehyde levels might be, at least in part, reflecting the oxidative stress at myocardium level. Nevertheless, it is pertinent to consider that malondialdehyde concentration in the plasma is most probably influenced by: poorly perfused peripheral tissues (i.e. skeletal muscles, guts, kidneys), cells within blood vessels besides the myocardium [2,16] and associated with increased metabolic rate [17]. This assumption is validated by the significantly higher concentrations of malondialdehyde found in the plasma compared with the levels in the pericardium.

In conclusion, we considered that the assessment of a simple malondialdehyde assay in plasma could give a reasonable approximation of the oxidative stress at pericardium level and that this parameter can be used as an early marker of ventricular dysfunction.

The limitations of this study were: (a) the absence of a control group for pericardium samples, because no patients submitted to cardiac surgery in our institution could accomplish this condition due to same extent of clinical, hemodynamic or cardiac dysfunction, (b) unknown origin of plasma or pericardial fluid malondialdehyde; (c) it is possible that administration of general anesthesia could affect the levels of oxidative stress [18]; however, this procedure was constant and the same for all the situations and patients studied. Therefore, an eventual influence on the correlations encountered seems unlikely but needs to be clarified in future studies and (d) the patients studied had different cardiac pathologies and relatively well preserved ventricular function, therefore, whether our findings will apply to patients with other conditions or more severe ventricular dysfunction requires further research.

Acknowledgements

This work was supported in part by grants FONDECYT 1010992, FONDAP 15010006 and Apertus-Andromaco.

References

[1] McMurray J, Chopra M, Abdullah I, Smith WE, Dargie HJ. Evidence of oxidative stress in chronic heart failure in humans. Eur Heart J 1993;14:1493–8.
[2] Keith M, Geranmayegan A, Sole M, Kurian R, Robinson A, Omran AS, et al. Increased oxidative stress in patients with congestive heart failure. J Am Coll Cardiol 1998;31:1352–6.
[3] Ghatak A, Brar M, Agarwal A. Oxygen free radical system in heart failure and therapeutic role of oral vitamin E. Int J Cardiol 1996;57:119–27.
[4] Fujita M, Komeda M, Hasegawa K, Kihara Y, Nohara R, Sasayama S. Pericardial fluid as a new material for clinical heart research. Int J Cardiol 2001;77:113–8.
[5] Díaz-Araya G, Naranjo L, Godoy L, Squella J, Letelier M, Nuñez-Vergara L. Antioxidants effects of 1,4-dyhidropyridine and nitroso aril derivatives on brain cerebral slices. Gen Pharmacol 1998;31:385–91.
[6] Misra H, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 1972;247:3170–5.
[7] Beers R, Sizer I. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J Biol Chem 1952;195:133–40.
[8] Paglia D, Valentine W. Studies on quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967;70:158–69 [of adrenomedullin: a natriuretic peptide. Am J Physiol 1995;268:F657–63.].
[9] Page E, Upshaw-Earley J, Goings G. Permeability of rat atrial endocardium, epicardium, and myocardium to large molecules: stretch-dependent effects. Circ Res 1992;71:159–73.
[10] Cheng W, Li BS, Kajstura J, Li P, Wolin MS, Sonnenblick EH, et al. Stretch-induced programmed myocyte cell death. J Clin Invest 1995;96:2247–59.

[11] Dhalla AK, Hill MF, Singal PK. Role of oxidative stress in transition of hypertrophy to heart failure. J Am Coll Cardiol 1996;28: 506–14.
[12] Kinugawa S, Tsutsui H, Hayashidani S, Ide T, Suematsu N, Satoh S, et al. Treatment with methylthiourea prevents left ventricular remodeling and failure after experimental myocardial infarction in mice: role of oxidative stress. Circ Res 2000;87:392–8.
[13] Castro PF, Diaz-Araya G, Nettle D, Corbalan R, Perez O, Nazzal C, et al. Effects of early decrease in oxidative stress after medical therapy in patients with class IV congestive heart failure. Am J Cardiol 2002; 89:236–9.
[14] Perez O, Castro P, Diaz-Araya G, Nettle D, Moraga F, Chiong M, et al. Persistence of oxidative stress after heart transplantation: a comparative study of patients with heart transplant versus chronic stable heart failure. Rev Esp Cardiol 2002;55:831–7.
[15] Mallat Z, Philip I, Lebret M, Chatel D, Maclouf J, Tedgui A. Elevated levels of 8-*iso*-prostaglandin $F_{2\alpha}$ in pericardial fluid of patients with heart failure. Circulation 1998;97:1536–9.
[16] Singh N, Dalla AK, Seneviratne C, Singal PK. Oxidative stress and heart failure. Mol Cell Biochem 1995;147:77–81.
[17] Poehlman ET, Scheffers J, Gottlieb SS, Fisher ML, Vaitekevicius P. Increase resting metabolic rate in patients with congestive heart failure. Ann Intern Med 1994;121:860–2.
[18] Yesilkaya A, Ertug Z, Yegin A, Melikoglu M, Baskurt OK. Deformability and oxidant stress in red blood cells under the influence of halothane and isoflurane anesthesia. Gen Pharmacol 1998;31:33–6.